INVESTIGATION ON THE LOCUS OF ACTION OF DDT IN FLIES (DROSOPHILA)

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INTRODUCTION

The experiments reported herein were designed to gain information as to where DDT produces its poisonous effect in the insect.

DDT poisoning in insects is characterized by symptoms of hyperactivity and discoordination of neuromuscular system, followed by convulsions and terminating in death. Isolated legs of DDT treated roaches continue to twitch after complete separation from the body, and isolated legs of normal roaches were induced to twitch by the application of DDT to the cut surface, although they remained quiescent if DDT was not applied (Yeager and Munson, 1945). Likewise the isolated legs of adult blowflies (*Phormia regina*) showed twitching movements when dipped before or after they were cut off from the animal into a one per cent DDT acetone solution, while untreated isolated legs remained motionless (Chadwick, 1945). From somewhat different experiments, Tobias *et al.* (1945) working with roaches suggested that the thoracic ganglia were the critical loci for the action of DDT. From these observations it appeared likely that the symptoms of DDT poisoning in insects resulted from an effect on the central nervous system, but that there existed also peripheral components which were not as yet exactly delimited.

MATERIAL AND METHODS

The experiments were performed on the larvae and adults of the fruit-fly (*Drosophila virilis*). The DDT preparation used was in form of an emulsion, (one per cent DDT, one per cent lecithin, ten per cent peanut oil, emulsified in a 0.95 per cent NaCl solution). This emulsion was injected by means of a micropipet into the abdominal cavity of the insect. The physiological saline solution used throughout the investigation was a Ringer solution modified for Drosophila (H_2O, 1000 cc.; NaCl, 7.5 gm.; KCl, 0.35 gm.; $CaCl_2$, 0.21 gm.). The various concentrations of phenobarbital used were also always made up in this Ringer solution. Imaginal discs were transplanted with the usual Drosophila transplantation technique.

EXPERIMENTAL

Behavior of larvae and adults after poisoning

When DDT emulsion was injected into the abdomen of adult flies which had been slightly narcotized with ether, the response to the poison was immediate. Legs and wings at once went into violent, uncoordinated movements. About twenty seconds after the injection the abdomen, previously motionless, began to

convulse; its movements were a rapid succession of short, uncoordinated spasmodic twitches. At first the convulsions were strong and a great deal of the injected emulsion was thus pressed out through the puncture wound. About five minutes after the injection the legs and wings went into a spasm and took up a characteristic position. The legs were drawn toward the body and crossed over ventrally, while the wings were folded backward. This position was maintained until the animal died. The contractions of the abdomen continued for about four to seven hours but became gradually weaker. After seven hours the animal was apparently dead. Although during all of this time the legs and wings remained in their spasmodic condition, one occasionally observed slight twitches of the tarsal segments and of the antennae; the wings, however, showed no movement. It was thus clear that the muscular response to the poison varied in different regions of the body, for the wing and leg muscles soon went into contraction and remained that way, while the muscles of the abdominal wall continued to convulse for a long period.

When an emulsion prepared in the same manner, but containing no DDT, was injected into flies, no effect was noted. The animals recovered from narcosis in the usual way, and were still alive the following day without any apparent injurious effects. Thus the symptoms described above were due to DDT and were not caused by the emulsion itself.

Larvae narcotized with ether were motionless, except for the pulsating heart tube visible through the transparent skin. When such larvae were injected with DDT emulsion, one observed at first a great acceleration of the heart-beat. Convulsions of the body wall began about twenty seconds after the injection. It was difficult to observe the heart-beat while the convulsions were in progress, but it was found in ligatured larvae, which will be described below, that the heart-beat soon became normal again after the initial acceleration. The larval convulsions were very strong and uncoordinated. Contraction wave after contraction wave passed over the creature, somewhat resembling crawling movements, yet the animal was unable to move from its place. The forward and backward movements were much more rapid than the normal crawling movements. Moreover, the animal never extended to its full length but remained partly contracted all the time. Short twitching contractions occurred in various parts of the body, and broke the wave-like contraction into a complex, uncoordinated movement. The larvae moved continuously in this way, some of them for twenty or more hours. The majority of such larvae died within ten hours, but some of them lived for twenty-thirty hours after the injection. The symptoms were the same, whether last (3rd) or late 2nd instar larvae were used for the experiments.

In control experiments, where emulsion containing no DDT was injected, no such symptoms occurred.

Ether Narcosis and DDT Symptoms

Normal flies etherized only until their movements stopped were completely relaxed if removed at that time from the ether. Their wings were in normal resting position and their legs were bent in the way assumed by flies at rest. Such flies recovered about one half hour after their removal from the anaesthesia and then seemingly behaved normally. Yet flies left for a longer time in the etherizer behaved quite differently. Their wings were folded back and their legs were

stretched out and held stiffly away from the body.   Usually flies thus over-etherized
did not recover from the narcosis and died in that position.

The reaction of these differently etherized flies to DDT poisoning was quite
interesting.   The slightly etherized individuals showed, after DDT injection, the
typical DDT symptoms described in the foregoing section.   On the other hand,
when over-narcotized flies were injected with DDT, one noticed that their legs and
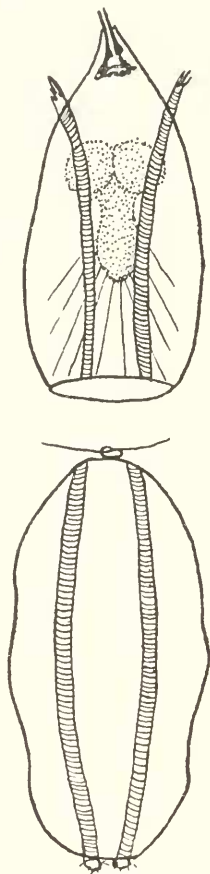

FIGURE 1. Diagram illustrating larval ligature experiments for separating the central
nervous system from the rear part of the body. Note location of central nervous system
(stippled) in anterior part.

wings did not respond to the poison.   Since such flies never recovered from the
narcosis it might be thought that they were already dead at the time of the DDT
injection.   This, however, was not the case, for their abdomens showed the typical
DDT convulsion.   These convulsions continued for about three hours, but were
somewhat weaker than those of the slightly etherized animals.   About that time the
uninjected but over-etherized control animals were still completely immobile and
apparently dead.   In comparing the uninjected and injected flies, one gained the
impression that the DDT treatment in some way partly released the ether block.

The Importance of the Central Nervous System

The central nervous system of Drosophila is concentrated in the anterior part of the body. In the larva the central nervous system is located in the third thoracic and the first abdominal segments; it consists of the two brain hemispheres, to which is attached a large ganglionic mass (Figs. 1 and 3). This large ganglion is a compound structure, for it includes the sub-oesophageal ganglion, the three thoracic ganglia, and the eight abdominal ganglia. In the adult insect the three thoracic ganglia are separated, but all eight abdominal ganglia unite into one ganglionic mass which extends into the first segment of the abdomen (Fig. 2).
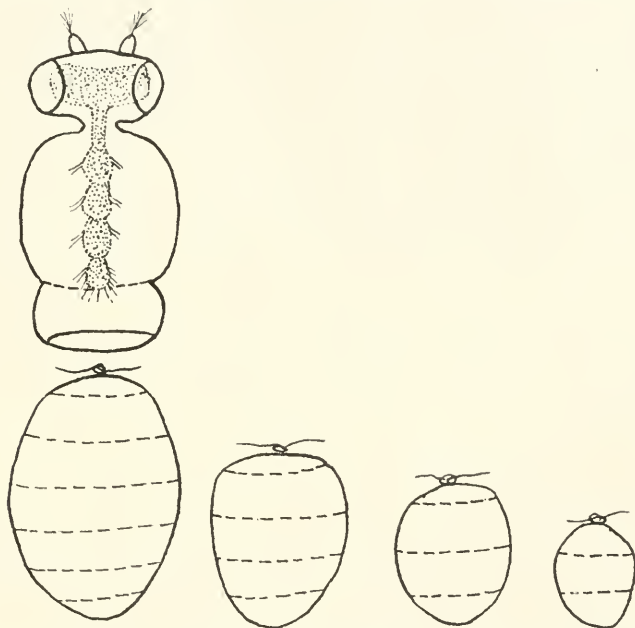

FIGURE 2. Diagram illustrating ligature experiments on adult flies, showing the separation of progressively smaller abdominal parts from the anterior region of the animal. Note location of central nervous system in anterior part of fly.

The described topography allowed one to separate experimentally large parts of the insect body from its central nervous system. The separation was accomplished by means of a fine silk ligature tied around the body of the animal. Depending upon the position of the ligature, smaller or larger parts of larvae and adults were thus isolated. In all experiments of this kind, the part in front of the ligature was cut away, in order to be sure that no connection to the ganglia remained (Figs. 1 and 2). Abdominal parts isolated in this way were completely motionless and stayed alive for several days.

The most useful symptom for the experimental approach was the contraction of the abdominal wall muscles under the influence of DDT. This effect was very uniform, clearly observable, and lasted a considerable time. The question then arose: were these movements under the control of the central nervous system?

In other words, was the muscular activity caused by an effect of DDT on the central nervous system? To test this possibility, isolated portions of larval and adult abdomens containing no ganglia were injected with DDT emulsion. The response to the injection was called forth immediately. Both larval as well as adult abdomens exhibited the same typical movements that were observed in the abdomens of injected intact flies. Again, as in the intact animals, it was found that the isolated adult abdomen responded somewhat faster than the isolated larval abdomen. That is, the larval abdomen responded at about thirty-sixty seconds and the adult abdomen about twenty seconds after the injection. The DDT convulsions of the isolated parts continued for a considerable time, but not as long as in the intact animals. In the isolated adult abdomen the convulsions clearly became weaker two hours after injection, yet weak contractions were observed five hours after the injection. In the isolated larval abdomens the contractions were still strong four hours after the injection, yet these parts never survived for twelve hours as injected whole larvae commonly did.

A similar sequence of events was observed when only the distal two or three segments of the adult abdomen were isolated from the rest of the body and then injected with DDT emulsion (Fig. 2) or when only two or three segments from the middle of the larval abdomen were isolated by means of two ligatures and then injected.

These findings showed that the central nervous system did not control the abdominal symptoms produced by DDT, since they occurred also in the absence of the central nervous system. However, it must be recognized that this does not imply that the central nervous system was unaffected by DDT.

Body Fragments and DDT Symptoms

In order to reduce the structural complexity of the insect body, in an effort to localize more closely the site of action of DDT, the following fragmentation experiments were performed. Rectangular pieces of skin were cut from the dorsal, lateral, or ventral wall of adult flies and placed in a drop of saline solution. These pieces, about two segments wide, included some muscles of the body wall, fat tissue, tracheae, and nerves but no ganglia. The pieces of dorsal body wall included also part of the heart tube and some alary muscles. In saline solution these pieces remained motionless, but if a few drops of DDT emulsion were added, the isolated muscles in the piece began to twitch. This response began about thirty seconds after DDT was added and continued for about two or three minutes. In control experiments where emulsion containing no DDT was added, no such response occurred.

These experiments confirmed those above by showing that the central nervous system was not necessary for the response of the abdominal muscles to DDT. It would appear that DDT affected either the muscles directly or the peripheral nerves. One way of distinguishing between these two possibilities would be to test a nerve-free muscle preparation with DDT. Technically, however, it is impossible to obtain such a preparation. Other methods were sought to settle this question and are described below.

Treatment With Phenobarbital

Phenobarbital is a drug which depresses central nervous activity in vertebrates. The effect of this substance on flies has not been described previously. When a ten per cent phenobarbital saline solution was injected into the abdomen of adult flies the animals were apparently killed instantly. The slight vibrating movements characteristic of lightly etherized flies stopped immediately after the injection. All of the muscles seemed to relax and the legs and wings were held in normal position.

Flies which had just come out of ether narcosis but were still sluggish lost their coordination when injected with a one per cent phenobarbital solution. Wings, legs, and abdomen moved uncoordinatedly for several hours until the animal finally died. The movement of the abdomen, also uncoordinated, was very different from the symptoms produced by DDT.

Injection of 0.1 per cent phenobarbital solution into adult flies induced narcosis, followed by complete recovery of the animal. One hour after the injection some flies crawled about slowly, while others were still unable to hold themselves up and fell over from time to time. By this time the effect of the ether had worn off and these symptoms were regarded as phenobarbital effects. One-half hour later the coordination of the animals had improved but their movements were still slow. But the next day the animals had recovered and behaved normally.

That phenobarbital affects the nerves rather than the muscles in flies was indicated by the following experiments. Fully grown larvae were split open along the mid-dorsal line. The intestine, Malipighian tubes, fat body and the main tracheae were then removed. Care was taken not to disturb the brain and ganglia in the anterior part of the body. This manipulation was carried out in physiological salt solution. The skin with its muscular layer and the adhering nervous system was placed in a small dish with a wax bottom and covered with fresh physiological salt solution. The preparation was then stretched out and by means of fine pins was fastened to the wax bottom of the culture dish, as shown in Figure 3. In this condition the tissues stayed alive for several hours. From time to time the muscles contracted, showing the typical wave-like contraction pattern of a crawling larva. After these movements had been observed for ten minutes the physiological solution was removed and replaced by a one per cent phenobarbital solution. The movements in the preparation stopped immediately. Again, after about ten minutes during which time no movements occurred the phenobarbital solution was removed and replaced by physiological saline. This solution was in quick succession drawn off and replaced about two or three times. The preparation was thus washed clean of any phenobarbital. It was now observed that normal movements had returned.

Ten minutes later the physiological solution was removed and replaced by one per cent phenobarbital solution; all movements again stopped. When washed and placed in physiological solution again the movements in the preparation reappeared. This procedure was repeated three or four times at intervals of about ten minutes and still the tissues continued to contract spontaneously when in physiological solution.

These experiments indicate that the one per cent phenobarbital solution was apparently not injurious to the tissues for the length of exposure tested. Moreover, while in phenobarbital, no muscular movement was observed, yet if the

muscles were stimulated directly by touching them with a fine needle, localized contraction in the region of the stimulus was noted. Following the mechanical stimulus the activated muscles contracted rather rapidly, stayed in the contracted state for some time and relaxed very slowly. This localized response of the muscles to mechanical stimuli when under phenobarbital indicates that the drug had not paralyzed the muscles directly but rather the nerves.
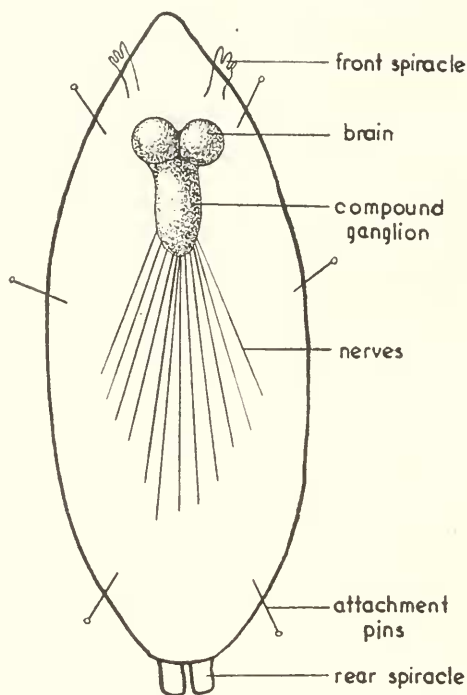

FIGURE 3.   Diagrammatic representation of the skin muscle preparation of a whole larva, pinned to the wax bottom of the culture dish.

Combined Phenobarbital and DDT Treatment

The knowledge that phenobarbital apparently acted on the nerves but not on the muscles provided a tool for determining whether DDT affects the nervous tissue or the muscles. If DDT could affect the muscles directly DDT symptoms should be provoked in animals paralyzed by phenobarbital. If, on the other hand, DDT affects only the nerves, no DDT symptoms should occur in animals treated with phenobarbital. That the latter is the case was shown by the following experiments.

In physiological solution the muscle preparation (Fig. 3) showed spontaneous crawling movements as described above. If a small amount of DDT emulsion was dropped onto such a preparation, the rhythm of the movements was interrupted; it became uncoordinated rapidly and resembled the movements observed in DDT-treated larval abdomens. If, on the other hand, DDT emulsion was dropped in the same manner onto preparations kept in one per cent phenobarbital solution, no

movement whatsoever took place. Moreover, if the physiological solution containing DDT emulsion was washed off from the actively moving preparation, and was replaced by one per cent phenobarbital solution, the movements ceased immediately. However, it was impossible to bring the preparation once treated with DDT back to its normal way of movement by washing the DDT solution off and replacing it with pure physiological solution. Such preparations still showed DDT symptoms. Apparently it was impossible to wash all the DDT out by the methods used. The DDT symptoms were of course stopped by placing the preparation again into phenobarbital.

Similar results were obtained when DDT-treated larval or adult abdominal parts were injected with one per cent phenobarbital solution. The DDT symptoms of such parts ceased immediately after phenobarbital was administered by injection. The reciprocal experiment, where phenobarbital was injected first, yielded the same results, for when DDT was injected into such treated abdomens no DDT symptoms occurred. The symptoms of whole larvae or adults which had been treated with DDT were also stopped immediately by injecting a one per cent phenobarbital solution.

Certainly the effects of phenobarbital on isolated, DDT-treated abdomens indicate that the absence of the central nervous system did not limit the action of the drug, showing that in insects phenobarbital may act on the peripheral nerves.

At this point the discussion of the phenobarbital effects on DDT poisoning must be augmented by an experiment showing how phenobarbital in weaker concentrations caused at least a partial recovery from DDT poisoning. It has been stated before that the legs and wings of adult flies injected with DDT were completely paralyzed five minutes after the injection. Now, when 0.1 per cent phenobarbital solution was injected into such animals, the movements of their legs were restored. These movements were uncoordinated and were similar to those observed in the beginning stages of DDT poisoning before the organism went into spasm. Also, the wings were able to move somewhat and were not folded backward. Even the convulsions of the abdomen were much less pronounced. Leg movements continued for about two hours, which of course was a much longer time than ever noted in animals injected with DDT only. These findings clearly show the antagonistic effect of phenobarbital on DDT.

CAPACITY OF DIFFERENT TISSUES FOR GROWTH AND DIFFERENTIATION AFTER DDT POISONING

If it is true, as the experiments seemed to indicate, that DDT affects only the nervous system, one might expect other tissues to be largely unharmed by the DDT treatment. This expectation can be tested experimentally. It is known (Bodenstein, 1943) that larval tissues will grow and differentiate normally when transplanted into the abdomen of larval or adult flies. In the larvae the transplanted tissues will develop in synchrony with their hosts to imaginal completion, and in adult flies, the transplant will undergo a considerable amount of growth. The capacity of the tissue for growth and differentiation, it is believed, offers a good criterion for testing the condition the tissue is in after being exposed to the poison. Tissues affected by DDT should certainly not develop normally. The following experiments were designed to clarify this issue.

Fifteen last-instar larvae were injected with DDT and placed on moist filter paper. Twenty-two hours later four larvae were still alive and showed typical DDT symptoms. Three of these larvae were opened and their eye discs, leg discs, and antennal discs dissected out. These discs are the primordia for the future eye, leg, and antenna of the adult fly.

They were transplanted into the abdomen of the mature larvae, one disc to each host. The several hosts carrying the transplants from one donor were kept separated from the hosts carrying the transplants of the other donors.

If the transplanted organs were able to develop normally, they should have been found as fully differentiated organs in the abdominal cavity of their respective hosts after metamorphosis. From the fifteen original hosts comprising the cases of all three series, eleven survived the operation. These animals completed their metamorphosis seven days after the operation and emerged. They were then dissected. Three completely differentiated legs, one eye, and one antenna supplied by the first donor were recovered. Hosts which received transplants from the second donor yielded three legs, two antennae, and two eyes, and from the third donor, three legs. All transplants were fully differentiated. As far as the detailed morphological differentiation of the tested organs was concerned, they were found to be completely normal, and there was no reason to believe that the histological differentiation likewise was not normal.

For another experimental series, five leg discs, dissected from the fourth living larva of the original set of DDT-treated animals, were transplanted into the abdomen of five adult flies. Each host in addition to the leg disc also received two ring glands. This structure is necessary for the continued growth of the transplant, for it furnishes a growth-promoting hormone (Bodenstein, 1943). Three days after the operation one host was killed and the leg disc dissected. It had grown but little. Three other hosts were killed six days after the operation and the transplants dissected. These leg discs had clearly become larger. Finally the last host was killed 24 days after the operation and the disc dissected. In this case the transplant had grown considerably and had reached an advanced state of differentiation. The results of this series of experiments were very similar to those obtained in transplanting normal discs in the same manner (Bodenstein, 1939 and 1943).

In conclusion, these two experimental series show that exposure to DDT for twenty hours in no way affected the capacity of the imaginal tissues for growth and differentiation. Hence these findings are further evidence that the nervous system alone is affected by DDT.

SUMMARY

1. The larvae and adults of *Drosophilla virilis* were fatally poisoned by injecting a one per cent DDT emulsion into the abdominal cavity. The poison produced a typical pattern of symptoms.

2. The neuromuscular system of the wings and legs was apparently very sensitive to the poison, for they went into spasm long before the muscles of the abdominal wall. There was also a difference in sensitivity to the poison between the larva and adult, the larva being more resistant to the DDT emulsion.

3. Phenobarbital was found to affect the nervous system. Paralysis by phenobarbital was also produced in the absence of the central ganglia. This shows that the drug also affected the peripheral nerves. Muscles of larvae treated with phenobarbital responded to mechanical stimulation.

4. Since DDT produced no symptoms in animals treated with phenobarbital and since animals treated with DDT lost their DDT symptoms when injected with phenobarbital, it was shown that DDT acted on the nervous system. Moreover, body parts which had been isolated from the central nervous system and then treated with DDT stopped convulsing after phenobarbital administration. This shows that DDT affected the peripheral nerves.

5. The methods used do not allow one to determine what part of the peripheral nervous system might be affected. There are three possibilities. The poison might affect (1) the motor nerves leading to the periphery; (2) the myoneural junctions; (3) the peripheral nerve net. It is however still questionable whether such a nerve net exists in *Drosophila*.

6. The antagonistic effect of phenobarbital on DDT was clearly indicated by the fact that the spasm of the legs and wings in DDT-treated flies was partly relieved by treatment with phenobarbital.

7. The conception that only the nervous system is affected by DDT has been strengthened by the fact that larval organs (imaginal discs) which had been exposed to DDT for twenty hours grew and differentiated normally when transplanted into untreated larvae.

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